



REVIEW

Reproducibility2020: Progress and priorities [version 1; referees: 2 approved]

Leonard P. Freedman ¹, Gautham Venugopalan², Rosann Wisman¹

¹Global Biological Standards Institute, Washington, DC, 20036, USA

²Gryphon Scientific, LLC, Takoma Park, MD, 20912, USA

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Abstract

The preclinical research process is a cycle of idea generation, experimentation, and reporting of results. The biomedical research community relies on the reproducibility of published discoveries to create new lines of research and to translate research findings into therapeutic applications. Since 2012, when scientists from Amgen reported that they were able to reproduce only 6 of 53 “landmark” preclinical studies, the biomedical research community began discussing the scale of the reproducibility problem and developing initiatives to address critical challenges. Global Biological Standards Institute (GBSI) released the “Case for Standards” in 2013, one of the first comprehensive reports to address the rising concern of irreproducible biomedical research. Further attention was drawn to issues that limit scientific self-correction, including reporting and publication bias, underpowered studies, lack of open access to methods and data, and lack of clearly defined standards and guidelines in areas such as reagent validation. To evaluate the progress made towards reproducibility since 2013, GBSI identified and examined initiatives designed to advance quality and reproducibility. Through this process, we identified key roles for funders, journals, researchers and other stakeholders and recommended actions for future progress. This paper describes our findings and conclusions.

Open Peer Review

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Corresponding author: Leonard P. Freedman (Lfreedman@gsbi.org)

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Introduction

Introduction and purpose of the report

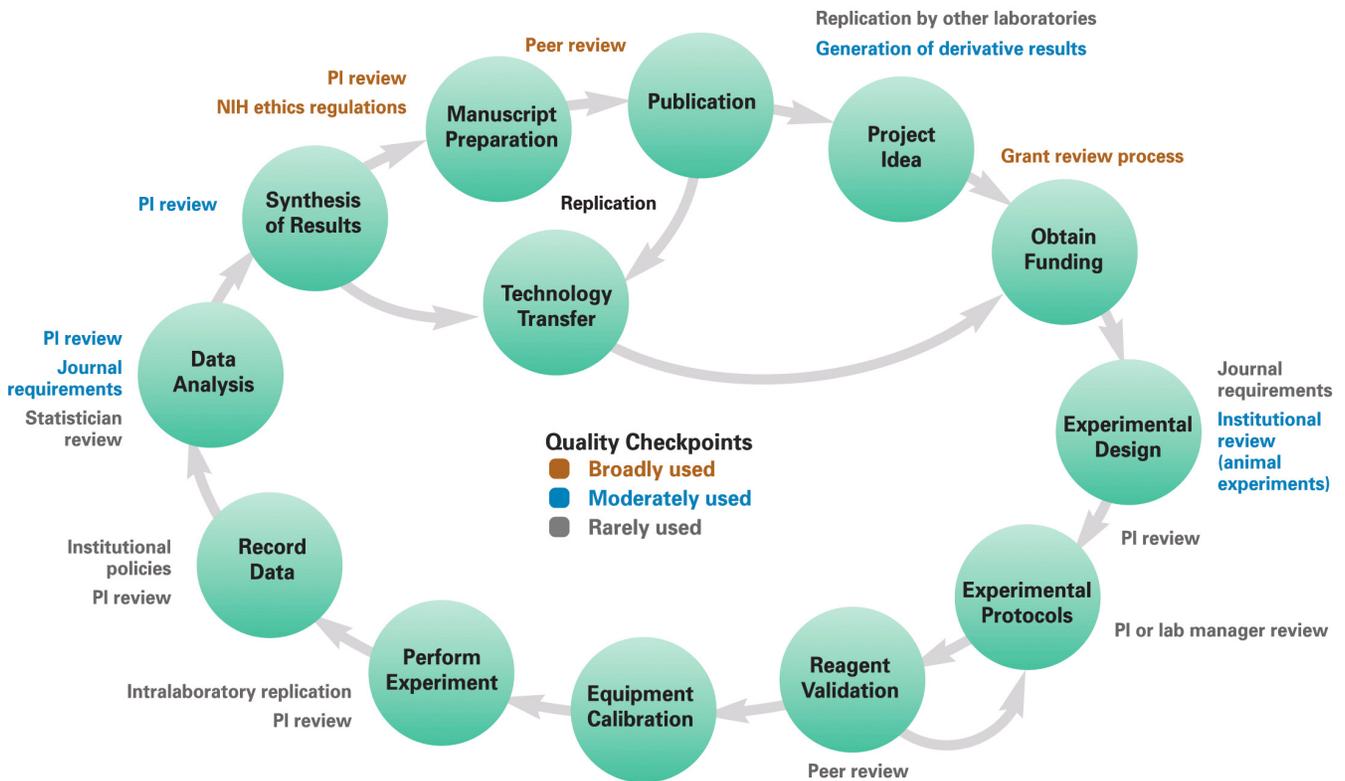
Preclinical biomedical research is the foundation of health care innovation. The preclinical research process is a cycle of idea generation, experimentation, and reporting of results (Figure 1)¹. The biomedical research community relies on the reproducibility of published discoveries to create new lines of research and to translate research findings into therapeutic applications. Irreproducibility limits the translatability of basic and applied research to new scientific discoveries and applications.

Although quality control during the research process centers on review of proposals and completed experiments (Figure 1), opportunities to improve reproducibility exist across the entire life-cycle of the research enterprise. In fact, as Figure 1 describes, there are very few steps in the cycle where quality check points are broadly used. By recognizing these opportunities, stakeholders, such as leading scientists, journals, funders, and industry leaders, are taking meaningful steps to address reproducibility throughout the research life-cycle, including commitments to scientific quality, a willingness to examine long-held research policies, and the development of new policies and procedures to improve the process of science.

The magnitude and effects of reproducibility problems are well documented. In 2012, scientists at Amgen reported that they were able to reproduce only 6 of 53 “landmark” preclinical studies².

Global Biological Standards Institute (GBSI) released the “Case for Standards” in 2013¹, one of the first comprehensive reports to address the rising concern of irreproducible biomedical research. Further attention was drawn to issues that limit scientific self-correction, including reporting and publication bias, underpowered studies, lack of open access to methods and data, and editorial and reviewer bias against publishing reproducibility studies (see Section IV)³. Based on these findings, GBSI completed an economic study in 2015 and estimated that the prevalence of irreproducible preclinical research exceeds 50%, with associated annual costs of approximately \$28B in the United States alone⁴.

Research community stakeholders have responded to these concerns with innovation and policy. In early 2016, GBSI launched the Reproducibility2020 Initiative to leverage the momentum generated by these stakeholder-led initiatives. Reproducibility2020 is a challenge to all stakeholders in the biomedical research community to improve the quality of preclinical biological research by the year 2020. The Reproducibility2020: Progress and Priorities Report (or Report), is the first to highlight progress and track important publications and actions, since the issue started to get broad research community and public attention in 2013^{5,6}. The Report addresses progress in the four major components of the research process: study design and data analysis, reagents and reference materials, laboratory protocols, and reporting and review. Moreover, the Report identifies the following broad strategies as integral to the continued improvement of reproducibility



Green circles indicate common steps in the life science research process. Adjacent color-coded text describes current/traditional quality checkpoints.

Figure 1. Many opportunities exist to improve reproducibility across the research life cycle. Figure from 1.

in biomedical research: 1) drive quality and ensure greater accountability through strengthened journal and funder policies; 2) engage the research community in establishing community-accepted standards and guidelines in specific scientific areas; 3) create high quality online training and proficiency testing and make them widely accessible; 4) enhance open access to data and methodologies.

Note to Reader: Terms such as reproducibility, replicability, and robustness lack consistent definition. The Report draws upon the definitions promulgated by the framework proposed by Goodman *et al.*⁷: “methods reproducibility” refers to the complete and transparent reporting of information required for another researcher to repeat protocols and analytical methods; “results reproducibility” refers to independent attempts to produce the same result with the same protocols (often called “replication”); and “inferential reproducibility” refers to the ability to draw the same conclusions from experimental data. The Report defines “reproducibility” to include issues affecting any of these three areas.

Irreproducibility: Drivers and impact

This report is organized around key areas in the life-sciences research process where action can significantly drive improved reproducibility⁴ (Figure 2):

- I. Study design and data analysis
- II. Reagents and reference materials
- III. Laboratory protocols
- IV. Reporting and review

The following sections contain detailed descriptions of each of these areas, including a review of the associated reproducibility problems, solutions, and examples of recent or current activities to promote greater quality and rigor (summarized in Table 1). The Report outlines the potential impact that lack of reproducibility has on the research community and its stakeholders (Table 2).

Methods

To identify key initiatives in reproducibility of biomedical research from 2013 to 2017, we conducted a review of literature, U.S. government policies, and online sources using the following keywords: reproducibility, rigor, transparency, and open access. Through these initial searches, we identified conferences on and funders of various efforts associated with reproducibility, which we used to identify other initiatives that were not identified using the keyword approach. We analyzed the information and developed recommended actions for promotion, and roles for life science stakeholders.

Results and discussion

I. Study design and analysis

Study design is the development of a research framework and analytical methods prior to beginning experiments⁸. A well-designed study has a research question with a rationale, and clearly defined experimental conditions, sample sizes, and analytic methods. In addition, researchers may include practices, such as blinded analysis, to mitigate subconscious bias. Pre-determining the research questions and sample sizes helps avoid problems such as “p-hacking” and selective reporting, where sample sizes and analytic variables are chosen based on their statistical significance rather than through a research framework (e.g., a hypothesis or an exploratory research model). Poor study design and incorrect data analysis can sabotage even a perfectly executed experiment.

Researcher surveys suggest that study design flaws are a key source of irreproducibility. Four of the top ten irreproducibility factors identified in a researcher survey relate to poor study design and analytical procedures¹⁰. These findings can promote a multi-faceted approach to improving study design and data analysis. Although researchers ultimately are responsible for ensuring sound study design and analysis, funder policies should encourage rigorous study design before research begins, journal requirements should facilitate better review of completed research, and training and support resources should improve researchers’ study design and analysis skills.

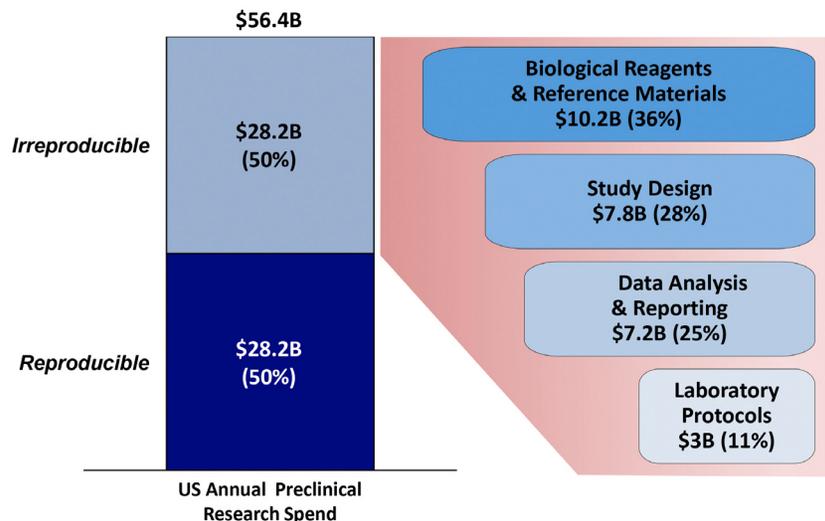


Figure 2. The magnitude of the reproducibility crisis and key sources of irreproducibility. Figure adapted from 4.

Table 1. Key sources of irreproducibility and solutions.

Source	Description of problem	Overview of solutions
Study design and analysis	Flawed study design and analysis introduce subconscious bias to data collection and reporting. Flawed study design and analysis is not captured in the p-value reported with a statistical data set, meaning the chance of an irreproducible finding is much higher than the commonly noted 5% threshold.	<ul style="list-style-type: none"> • Funder policies require grantees to clearly report study design and data analysis parameters • Journal guidelines establish baseline requirements to describe study design and analysis in manuscripts • Alternate review models to help verify study design • Courses, textbooks, and journal articles to build researcher capability • Statistical consulting services
Reagents and reference materials	Reagent variability between two different researchers (or the same researcher over time) introduces experimental variation. Key sources of variability include material variability and cell culture contamination/drift. Researchers often lack standards for commonly-used reagents. Where they exist, standards and verification are not always part of routine laboratory practice.	<ul style="list-style-type: none"> • Make cell line authentication and infection testing routine • Establish standards for commonly used reagents • Development of new technologies and verification strategies for key reagents • Reduce reliance on “black box” ingredients where possible. Characterize black box reagents where used
Laboratory protocols	Process variability across labs introduces results variability, even with validated reagents and reference materials. Descriptions of protocols in journals and on websites are often insufficient for results reproducibility. Tacit knowledge is difficult to obtain through written protocols.	<ul style="list-style-type: none"> • Protocol repositories facilitate transparency, sharing, and version control • Consensus minimum standard for methods sections in journal articles • More access to protocol videos to communicate tacit knowledge
Reporting and review	Lack of ready access to the data and manuscripts hinders post-publication review of new findings. Barriers to obtaining, analyzing and communicating decrease the community’s ability to identify and appropriately respond to flawed research.	<ul style="list-style-type: none"> • Enhanced reporting guidelines for scientific publications • Open access policies from funder and related support services and training for grantees • Data standards facilitate analysis and comparison of data sets from separate studies • Availability of funding and publication opportunities for results reproducibility studies incentivizes researchers to conduct them • Online forums and science journalism facilitate discourse and situational awareness

NIH study design policy. Funder policies that require good study design are especially powerful because they encourage researchers to develop rigorous study plans before beginning experimentation. Clinical research has regulatory mechanisms to review study design; for example, Phase 2 and 3 Investigational New Drug clinical trial applicants must acquire FDA approval of the study design and statistical analysis plan that includes explicit description of contingencies, such as sample exclusion criteria (<http://www.accessdata.fda.gov/SCRIPTs/cdrh/cfdocs/cfCFR/CFR-Search.cfm?CFRPart=312>). Preclinical biomedical research is not covered by these regulatory standards, and generally has not required explicit justifications of key parameters, such as sample sizes and statistical tests, in the hypothesis and specific aims sections of proposals or in publications. For example, an analysis of 48 neuroscience meta-analyses found that 28 (57%) of the studies had a median study power of 30% or less, despite the

relative ease of increasing sample size¹¹. The new NIH policy (see **Box 1**) requires grant reviewers to explicitly incorporate several key rigor and transparency features into their peer reviews, but the policy does not add dedicated scoring line items for these areas. With respect to study design and analysis, the policy requires grant applicants to evaluate the rigor of prior studies that form the basis of a research proposal, and to justify their proposed study design. In the first round of reviews with the new guidelines, the NIH Center for Scientific Review noted that panels increasingly discussed the areas of emphasis, but that additional communication is required to get all reviewers and applicants on the same page (<http://www.csr.nih.gov/CSRPRP/2016/09/implementing-new-rigor-and-transparency-policies-in-review-lessons-le>). Formal evaluations of this ongoing effort will provide valuable lessons for NIH and other funders interested in implementing their own rigor and transparency guidelines.

Table 2. Reproducibility affects all stakeholders in preclinical life sciences research.

Stakeholder	Implications of irreproducibility
Funders	<ul style="list-style-type: none"> • Impeded progress towards achieving organizational mission and goals • Wasted resources spent on funding follow-on research based on a flawed premise • Inefficient use of resources spent on checking, correcting, and refuting irreproducible work
Researchers and Research Institutions	<ul style="list-style-type: none"> • Adverse effect on reputation and career prospects • Difficulty in obtaining future funding • Failure of research projects that are based on irreproducible findings from the literature
Journals	<ul style="list-style-type: none"> • Impact of irreproducibility could negatively affect reputation, readership and journal prestige • Increased administrative costs of managing retractions and errata
Industry	<ul style="list-style-type: none"> • Expensive failed clinical trials • Resources wasted on failed in-house results reproduction • Decreased trust in providers' products leading to decreased sales
Nonprofits/Scientific Societies	<ul style="list-style-type: none"> • Unrealized opportunities to provide value to stakeholders and members in line with organizational mission
Public	<ul style="list-style-type: none"> • Delayed realization or lost opportunities of health care benefits based on preclinical research findings, negatively impacting the discovery of life-saving therapies and cures • Inefficient spending of taxpayers' money

To augment these efforts, NIH has worked with the journal community to develop publication guidelines (see Section IV), and funded the development of researcher training programs in study design (see “Training and Support” below) as part of its rigor and reproducibility efforts.

Box 1. Strengthened funder policies

As the largest and most influential research funder in the world, NIH took a major step in establishing new guidelines and going on record that NIH will address other areas where they can impact reproducibility⁹. NIH serves as an important model for other government and private research funders looking to establish greater accountability around quality and rigor.

NIH Rigor and Transparency Guidelines

NIH's Rigor and Transparency Guidelines went into effect on January 25, 2016 (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html>, <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-012.html>) This policy includes applicant and reviewer guidance in four key areas: scientific premise, scientific rigor, consideration of sex and other biological variables, and authentication of key biological and/or chemical resources (https://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Transparency.pdf) Applicants are required to describe the strengths and weaknesses of prior studies cited in their scientific premise, specifically they are required to describe and justify the proposed study design, and develop authentication plans based on established standards. Since reviewers are now instructed to review applications based on these criteria, grant applicants that fail to meet the new criteria are less likely to be funded. NIH also requires grantees to report on rigor and transparency measures in their publications and the Research Performance Progress Reports submitted during the life of an award. These new guidelines underscore the need for development and propagation of study design training, pre-registration resources, and low cost authentication tools. For further information, see the NIH webpage: <https://grants.nih.gov/reproducibility/index.htm>

Journal efforts to improve study design. Several studies indicate that fewer than 20% of highly-cited publications contain adequate descriptions of study design and analytic methods¹². At least 31 journals have signed on to the Principles and Guidelines for Reporting Preclinical Research, which included a call for journals to include statistical analysis reporting requirements and to verify the statistical accuracy of submitted manuscripts (see Section IV) (<https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research>). As these principles do not specify what these requirements should be, implementation varies by journal. One example from the *Biophysical Journal* recommends that authors consult with a statistician and requires reporting of specific information about sample sizes and statistical analyses (<http://www.cell.com/pb/assets/raw/journals/society/biophysj/PDFs/reproducibility-guidelines.pdf>).

In the United Kingdom, the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines developed by the National Centre for the Replacement Refinement & Reduction of Animals in Research, include a checklist for researchers who perform animal studies to help researchers appropriately report study design and sample size justifications (www.nc3rs.org.uk/arrive-guidelines). These guidelines can also be used to help ensure that researchers are planning their animal experiments correctly. As of January 2017, these reporting guidelines have been endorsed by nearly 1,000 journals and are required by the major funders in the UK, including the Wellcome Trust and Medical Research Council (<https://www.nc3rs.org.uk/arrive-animal-research-reporting-vivo-experiments>).

Some journals are prototyping alternate review models to help verify study design. As of January 2017, the Registered Reports initiative through the Center for Open Science allows selected reviewers to comment on study design and methods prior to data collection (<https://cos.io/rr>). Once study design has been

approved, participating journals essentially guarantee publication so long as the authors follow the study design. In addition, researchers can use the Registered Reports format to submit articles to these journals. Currently, 45 journals are participating in this initiative. In a separate, but related initiative, the Center for Open Science's Pre-Registration Challenge has been designed to provide training and incentives for up to 1,000 researchers to pre-register study protocols and submit manuscripts to participating journals (<https://cos.io/our-services/prereg/>).

One journal, *Psychological Science*, currently is pilot testing *statcheck* software on all submitted manuscripts (http://www.psychologicalscience.org/publications/psychological_science/ps-submissions). *Statcheck* and *StatReviewer* are tools developed by researchers to automatically review data analysis information contained in published manuscripts^{15,16}. Researchers also have broadly deployed the *Statcheck* tool on thousands of published studies (see Section IV).

Training and support. Many life-science researchers will require training and support to satisfy the funding and publication policies described above. In the 2016 Proficiency Index Assessment (PIA) (see **Box 2**), GBSI surveyed over 1,000 researchers of varying experience levels. Participants reported lower confidence in their skills in study design, data management, and analysis compared to their experimental execution skills¹³. Furthermore, research experience did not correlate with higher study design proficiency, suggesting the value of ongoing training and support in this area. New textbooks^{8,17}, online minicourses (<https://www.nih.gov/research-training/rigor-reproducibility/training>)¹⁸ and journal articles¹⁹ can be used for course development or independent study by more senior trainees.

Box 2. Online training and proficiency testing

New approaches to training researchers should be a priority for all steps in the research cycle, including the study design training resources described in the Report. Enhanced training should be available for all levels of researchers—graduate students, post-docs, and experienced PIs. Active learning opportunities are particularly important, considering the informal apprenticeship culture of science, in which trainees learn how to design, perform, and report on their research by working with more senior scientists. However, not all senior researchers have the most current expertise or may not be able to spend the requisite time with their trainees. Surveys of researchers support this need: the 2016 Proficiency Index Assessment indicated that even experienced researchers stand to benefit from study design training, and a *figshare* and *Digital Science* survey reported that over half of researchers wanted training on open access policies and procedures^{13,14}.

Innovative pedagogical approaches are required to ensure that training is effective and engaging for researchers at all stages of their careers. These approaches, including interactive teaching, in-lab practice, and proficiency assessments, are increasingly being explored by many institutions (see "Training and Support" example in Section I). Online training modules are a cost-effective way to provide high-quality, accessible, interactive training for researchers at all levels.

The positive response to study design courses established at Johns Hopkins University²⁰ and Harvard University (<https://nanosanothercourses.hms.harvard.edu/node/96>) demonstrate the value

of study design training. These courses are becoming more widespread and better tailored to the needs of life scientists, but are not universally available or required. Efforts are underway to increase the experimental design skillset of early-career students, but funding in this area has been relatively modest and in general, private funders have seen training and education as the responsibility of government funders and graduate programs. In 2014, NIH funded graduate courses on study design. Since 2014, NIH has issued a series of four funding opportunities for grantees interested in providing study design instruction for their graduate students and postdoctoral trainees through administrative supplements to existing grants (<https://www.nih.gov/research-training/rigor-reproducibility/funding-opportunities>, <https://grants.nih.gov/grants/guide/rfa-files/RFA-GM-15-006.html>). Several of these grantees have used the funds to develop study design training programs that are tailored to their respective research areas (<https://www.nigms.nih.gov/training/instpredoc/Pages/admin-supplements-prev.aspx>). For more computationally-focused researchers, a Harvard course on reproducible genomics is available online for free²¹.

In addition to training, researchers now have increased access to expert support during study design and analysis. University statistics departments often provide free consulting services to affiliated researchers (<http://statistics.berkeley.edu/consulting>, <https://catalyst.harvard.edu/services/biostatsconsult/>, <http://www.stat.purdue.edu/scs/>), and the Center for Open Science provides a similar service (<https://cos.io/our-services/training-services/>). The CHDI Foundation provides protocol and study design assistance, evaluation, and review to researchers studying Huntington's disease (<http://chdi-foundation.org/independent-statistical-standing-committee/>). This model may be of interest to other disease-specific funders as a low-cost investment that can improve research rigor and strengthen the community of practice in their mission area.

Together, these training and support resources work together to improve reproducibility by increasing the general standard of rigor for all research. As researchers gain an improved understanding and awareness of study design, they can design their own studies better and more effectively communicate with statistics consultants, conduct peer review, and evaluate published findings that may inform future work.

II. Reagents and reference materials

Reproducibility is difficult if labs are not working with the same research reagents and materials. Supplier-to-supplier variability often is poorly characterized until researchers run into problems with results reproducibility, as demonstrated by the example of synthetic albumin. The structure, stability, and immunogenicity of synthetic albumin varies across suppliers and lots, in ways that are not commonly characterized²². In addition, factors, such as lot-to-lot material variability, cell line drift, and contamination, can cause an individual researcher's assays to change over time. Examples from other sectors suggest that these problems can be addressed with standards.

Materials developed and validated based on standards are well-characterized and demonstrate consistency. Standardized materials that exhibit a predictable behavior can be used reliably in methods reproducibility, and can facilitate development of reference materials for assay validation. Standards of most well-known and often-used biological materials typically apply to particular

clinical applications, such as virus strains used in influenza vaccine development¹. Although preclinical researchers often use standardized chemical reagents (e.g., salts and sugars), few standardized biological materials exist. However, surveys suggest that life science researchers increasingly understand the need for standardized materials¹, and the research community recently has made progress on cell line authentication and antibody validation.

Standards development for biomedical research reagents. Stakeholders of preclinical research include researchers, reagent manufacturers, funders, journals, standards experts, and nonprofit organizations from countries throughout the world. Recent efforts to establish antibody databases, information-sharing requirements, and international frameworks for antibody validation standards are good examples of the broad, multi-stakeholder approach required to develop consensus standards around a specific reagent (see **Box 3**).

Box 3. Improved reagent standards: the Antibody Initiative

The research community has acknowledged that antibodies are an area of widespread error and inaccuracy²³. The Antibody Validation Initiative, involving stakeholders throughout the research community and led by GBSI, is an example that could be replicated in other scientific areas (e.g. both stem cells and synthetic biology are areas where a greater emphasis on development of standards and best practices are needed to ensure quality and advance discovery). Antibodies are key reagents in preclinical research for activities as diverse as protein visualization, protein quantification, and biochemical signal disruption. Antibody performance is variable, with differences in specificity, reliability, and functionality for different types of experiments (e.g., Western blotting and immunofluorescence), manufacturers, and lots, harming reproducibility²⁴. Stakeholder solutions include antibody databases, such as the CiteAB database (<https://www.citeab.com/>), and repositories, such as the proposed universal library recombinant antibodies for all human gene products²⁵. In all cases, validation is a key component of the solution.

NIH specifically highlights antibody authentication in the Rigor and Transparency guidelines, (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html>) providing additional impetus for new standards, policies, and practices. Researchers, manufacturers, pharmaceutical companies, funders, and journals have held dedicated conferences on antibody validation e.g. (<http://www.antibodyvalidation.co.uk/>). In 2016, the International Working Group on Antibody Validation (IWGAV) qualitatively identified key validation “pillars” that may be suitable for assessing antibody performance²⁶. Seeking to build on the IWGAV recommendations, GBSI and The Antibody Society organized a workshop for all stakeholder groups to develop actionable recommendations to improve antibody validation²⁷. Stakeholder groups recognized the shared responsibility of antibody validation and effective communication of validation methodology and results. In addition, they highlighted the need for continued, multi-sectoral engagement during the development of standards for validation, which may vary by use case, and information-sharing, which may vary by stakeholder.

Since the workshop, GBSI established seven multi-stakeholder working groups to draft validation guidelines for the major antibody applications. Validation guidelines will include an application-specific point system to quantify antibody specificity, sensitivity, and technical performance. The Antibody Validation Initiative also includes a Producer Consortium to address issues of common concern for producers and a Training and Proficiency Assessment program to ensure the highest quality of validation.

Good cell culture practice. One well-known example of developing standards for laboratory reagents is cell culture validation, which includes assay validation, cell line authentication, and testing for contamination²⁸. Many commonly-used cell lines are available from repositories, such as ATCC, as well as other nonprofit, governmental, and for-profit organizations. These organizations regularly test and validate the cells, confirming desired cell function and testing for accidental cross-contamination or infection. Researchers in two different labs can purchase validated cells from these providers and be assured that they are receiving the same product, but cells diverge once they are used in the lab. Use of shared sterile culture hoods, incubators, and reagent storage spaces can cause infection with bacteria, viruses, mold, or yeast, and result in unintentional cross-contamination of purchased cells with other cell cultures used in the lab. Even without contamination, genetic changes occur in cells through repeated culturing and experimentation, a process known as cell line drift. Despite these known problems, periodic cell line authentication and infection testing are not universally-practiced in preclinical research even though a human cell authentication standard exists^{29,30}.

As with study design, cell culture validation can be enhanced with policies from funders and journals. For example, the Prostate Cancer Foundation has been a leader in validation of cell lines used to study the disease, requiring periodic cell line authentication since 2013. NIH now requires grant applicants to describe their authentication plan as part of the Rigor and Transparency guidelines (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html>) and many journals now ask researchers to perform cell line authentication (<http://www.scoop.int/t/cell-line-contamination/p/4040895974/2015/04/08/which-journals-ask-for-cell-line-authentication>).

Many of the validation assays required for cell culture validation can be borrowed directly from other applications. In 2011 and 2012, ATCC organized an international group of scientists from academia, regulatory agencies, major cell repositories, government agencies, and industry to develop a standard that describes optimal cell line authentication practices, *ANSI/ATCC ASN-0002-2011*. The authentication assay uses Short Tandem Repeat (STR) profiling technology and is an affordable cell line authentication tool. The International Cell Line Authentication Committee’s *Database of Cross-contaminated or Misidentified Cell Lines* provides researchers with a dataset to check during the authentication process³¹. For products of animal origin, U.S. Department of Agriculture regulations specify testing protocols for mycoplasma and select viruses³² and test kits are commercially available.

Improving the reproducibility and translation of biomedical research using cultured cell lines must build on ongoing, multi-stakeholder efforts to raise awareness of the issues of misidentification and the role of authentication³³. GBSI’s #authenticate campaign encourages this kind of stakeholder engagement (www.gbsi.org/authenticate).

Technology and assay development. The development and propagation of standards is an iterative process. For example, recent publications highlight the simultaneous progress in cell line authentication technologies and standards development, including the establishment of reference data standards and cell line authentication policies for the broader research community^{28,29}. As technology development progresses, the standards need to be revisited and

improved to reflect the current capabilities afforded by new tools³⁴. For example, more affordable next generation sequencing is an increasingly useful tool to validate genome editing and characterize changes in cell behavior³⁵, and mass spectrometry and lab-on-a-chip assays can help characterize sera and other liquid reagents^{36,37}.

Sera validation: an opportunity for standards and technology development. One opportunity to further improve cell culture validation would be to develop standards for sera production and validation. The media used to feed most cells in culture include sera, such as fetal bovine serum, that provides a variety of growth factors and other small molecules. Even authenticated cells may perform very differently in two different sera preparations. Serum is a “black box” ingredient with high variability between manufacturers and lots. Recently developed best practices include characterizing and reporting information on the particular lot(s) of serum/sera used in an experiment, and repeating an experiment with multiple lots of sera to ensure that observed phenotypes are not serum-related artifacts³⁸. Serum manufacturers have begun to characterize and validate sera (<http://www.bioind.com/support/tech-tips-posters/introduction-to-fetal-bovine-serum-class/>), but no industry standard exists for reporting serum characteristics and reliability.

Further technological development could reduce reliance on sera. In serum-free culture, researchers precisely define all components of the cell culture medium rather than using a “black box” serum. Building a system with defined minimum essential components improves reproducibility and enhances scientific understanding of the key signaling molecules involved in biological processes of interest³⁸. Researchers are developing and validating robust, serum-free culture systems. Clear material and validation standards are building blocks that facilitate this development.

III. Laboratory protocols

Reproducibility requires thorough, detailed laboratory protocols. Without ready access to the original protocols, researchers may introduce process variability when attempting to reproduce the protocol in their own laboratories. The respondents of the GBSI's Proficiency Index Assessment were more confident in their experimental skills than their study design skills¹³. Despite this relative confidence in their laboratory execution skills, researchers frequently are unable to recreate an experiment based on the experimental methods published in journals, which usually do not contain step-by-step laboratory protocols that specify every relevant variable. Further, a particular study may use a modified version of an established protocol, but state the method was “as previously described” without noting the changes. If attempts to contact authors to request the original protocols are not successful, the reader may not be able to reproduce the methods in the published work. In a *Nature* survey, nearly half of researchers felt that incomplete experimental protocol descriptions in published articles hindered methods reproduction efforts¹⁰. Although fewer efforts exist in this key area than in the other three areas described in this report, newly developed tools and processes designed to facilitate protocol sharing and version control may improve documentation and reduce barriers to methods reproduction.

Protocol repositories. Protocol repositories are an innovative approach that may facilitate transparency, protocol sharing, and version control. Researchers can upload their protocols to a repository,

such as [Protocols.io](http://www.protocols.io), precisely specifying all step-by-step instructions with links to required reagents. As the original researchers, or others, modify the protocol, they can document these changes in the repository and create their own “forked” version of the protocol. Protocols in the repository can receive a DOI number, making identification of the precise version used in a publication easier. Suppliers also can post recommended protocols for their products on these websites, which facilitates adoption of their products.

Protocol development requires a robust community of practice, so that protocols can be developed and tested by researchers in different laboratories. This practice ensures that the written instructions are understandable and replicable by a third party. Emerging on-line tools, such as BioSpecimen Commons (The Bidesign Institute at Arizona State University), provides a common location and uniform set of protocols and conditions for clinical sample-related standard operating procedures. Another example is the international Protist Research to Optimize Tools in Genetics group, funded by the Gordon and Betty Moore Foundation, and working on the Protocols.io website ([https://www.moore.org/article-detail?newsUrlName=\\$8m-awarded-to-scientists-from-the-gordon-and-betty-moore-foundation-to-accelerate-development-of-experimental-model-systems-in-marine-microbial-ecology](https://www.moore.org/article-detail?newsUrlName=$8m-awarded-to-scientists-from-the-gordon-and-betty-moore-foundation-to-accelerate-development-of-experimental-model-systems-in-marine-microbial-ecology), <https://www.protocols.io/groups/protist-research-to-optimize-tools-in-genetics-protg>). As of January 2017, this group has 95 members who have contributed 31 protocols to the platform. Although this group does not focus on preclinical research, the practices established by this group are a relevant example that could be reproduced in preclinical research. Preclinical research funders may find added value with version control, protocol forking, and communities of practice in their areas of interest.

Improved protocol reporting in journals. The Principles and Guidelines for Reporting Preclinical Research also call for “no limit or generous limits on the length of methods sections.” (<https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research>) However, most methods sections still do not contain step-by-step protocols. Authors submitting to participating journals can include links to Protocols.io in the methods section, specifying the exact version of a protocol that was used in the study with a DOI number (<https://www.protocols.io/partners?publishers>). In April 2017, PLOS and Protocols.io announced a partnership where PLOS is encouraging their authors to log their experimental methods in Protocols.io (<https://www.moore.org/article-detail?newsUrlName=open-access-to-data-and-the-laboratory-methods>).

Although methods journals (i.e., those dedicated to publishing detailed methods) usually provide sufficient information about protocols, most scientific publications do not. Even new techniques are not described in full detail because they build on established techniques, the methods for which are not fully described. However, some journals, such as the *Journal of Visualized Experiments*, publish original, peer-reviewed manuscripts and videos of both established and new techniques (<http://www.jove.com/>). The use of videos helps to communicate technique subtleties that may not be captured in written instruction. This type of tacit knowledge often only can be obtained by visiting a laboratory and learning directly from the protocol developers.

IV. Reporting and review

The scientific community requires ready access to publications and the original underlying data to adequately review studies and conduct results for reproducibility efforts. Journal reporting guidelines improve methods reproducibility by ensuring that manuscripts contain a minimum standard of required information. Data standards further facilitate this process, as large data sets formatted in an agreed-upon, machine-readable format are easier to find, compare, and integrate across different studies. With better access to data and manuscripts, researchers now can engage in more robust post-publication review. Reducing these barriers can improve reproducibility by identifying potential flaws in published papers, making scientific self-correction and self-checking faster and cheaper.

Enhanced journal reporting guidelines. Journals increasingly recognize the importance of methods reproducibility and are developing more transparent and enhanced reporting guidelines. Co-led by the Nature Publishing Group, the American Association for the Advancement of Science (AAAS; publisher of *Science*), and the NIH (as part of its Rigor and Reproducibility efforts), the scientific journal community established the Principles and Guidelines for Reporting Preclinical Research in June 2014 (<https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research>). Per the last update of the NIH website in 2016, 31 journals have signed on to these guidelines (<https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research>). The guidelines provide a minimum consensus standard for statistical rigor, reporting transparency, data and material availability, and other relevant best practices, but do not specify in detail exactly what these reporting requirements should be.

More specific guidelines from journals have built upon this initial effort. Differences in implementation of reporting guidelines may cause some short-term confusion among authors and reviewers. However, over time, their implementation could provide long-term benefit in identifying successful approaches and best practices. One initiative that seeks to provide broad direction and even instruction to journals are the Transparency and Openness Promotion (TOP) Guidelines, promulgated by the Center for Open Science's Open Science Framework. TOP includes templates for journals interested in implementing their own reproducibility guidelines, and exist in a tiered framework so journals can gradually implement more stringent standards as they improve their own implementation and review capability³⁹. Several of the journals highlighted in the examples listed below are signatories to the TOP guidelines.

- Expanded reproducibility guidelines from the *Biophysical Journal* are an example of what enhanced journal guidelines look like in practice. These guidelines specifically establish reporting standards in four key areas: Rigorous Statistical Analysis, Transparency and Reproducibility, Data and Image Processing, and Materials and Data Availability (<http://www.cell.com/pb/assets/raw/journals/society/biophysj/PDFs/reproducibility-guidelines.pdf>).
- Authors submitting to the Nature Publishing Group family of journals must complete a reporting checklist to ensure compliance with established guidelines, including a requirement

that authors detail if and where they are sharing their data (<http://www.nature.com/authors/policies/checklist.pdf>).

- STAR Methods guidelines (Structured, Transparent, and Accessible Reporting) are designed to improve reporting across Cell Press journals. These guidelines remove length restrictions on methods, provide standardized sections and reporting standards for methods sections, and ensure that authors include adequate resource and contact information (<http://www.cell.com/star-methods>).
- Since January 2016, researchers funded by the Howard Hughes Medical Institute have been required to adhere to a set of publication guidelines that cover similar areas as the minimum consensus guidelines described above (http://www.hhmi.org/sites/default/files/About/Policies/sc_300.pdf).
- The Research Resource Identification Initiative establishes unique identifiers for reagents, tools, and materials used in experiments, reducing ambiguity in methods descriptions⁴⁰.

Journals and funders can use two methods to measure and continuously improve implementation of these guidelines: 1) stakeholder feedback studies; and 2) research measuring the frequency of compliance over time. The journal community periodically should reconvene and use data from these evaluations to identify and propagate successful implementation of the Guidelines, and to update and improve the Guidelines.

Open access policies. Funder policies increasingly mandate access to data and publications (see [Box 4](#)). As of October 2016, 16 U.S. government funding agencies require their grantees' publications to be open access within a year of the publication date, and 13 of these funders, including the NIH, require data management plans to be included in research proposals⁴¹. Globally, the online research repository *figshare* predicts that by 2020, all funders in the developed world will require openness¹⁴. At the end of March 2017, the European Commission (EC; institute of the European Union) expressed an interest to set up a "publishing platform" to stimulate open-access publishing in Europe⁴². The EC is hopeful the platform will catalyze their initial plan to make all published research funded by EU members open access by the year 2020 (<http://www.sciencemag.org/news/2017/03/european-commission-considering-leap-open-access-publishing>).

Private funders have taken a variety of approaches to promoting open access, such as increasingly requiring either full open access or archived manuscripts as a condition of continued funding (reference [<https://www.ucl.ac.uk/library/open-access/research-funders>] contains a summary of many institutions' policies). The Bill & Melinda Gates Foundation is a leader among philanthropic organizations in formulating and implementing open access policies. Beginning in January 2017, the Gates Foundation's Open Access Policy requires immediate open access ("Gold" access) for all publications and underlying data generated by authors that it supports (<http://www.gatesfoundation.org/How-We-Work/General-Information/Open-Access-Policy>).

Many journals already have open access options that comply with the Gates Foundation policy, but some high-profile journals such as *Nature*, and *Science*, did not have Gates-compliant policies as of January 2017⁴³. In response to this policy change, AAAS reached a provisional agreement with the Gates Foundation to make Gates-funded publications in AAAS journals open access⁴⁴. Similarly, the *Cell Press* family of journals has special agreements with a number of funders, including Gates, that allow immediate open access for a fee (<http://www.cell.com/rights-sharing-embargoes>). This issue warrants further attention as funders and journals continue to negotiate around access permissions. The Wellcome Trust has a similar policy, encouraging immediate open access but allowing a six-month delay. Both the Wellcome Trust and Gates Foundation have provided dedicated funding to support open access fees imposed by journals where appropriate, and prefer the unrestricted Creative Commons-BY license (<https://creativecommons.org/licenses/by/4.0/>). More recently, both the Gates Foundation and the Wellcome Trust took the additional step of partnering with F1000 to establish publishing platforms for their grantees.

While this represents real progress, these policies can be a source of confusion for researchers. In a recent survey of over 1,000 researchers by figshare and Digital Science, 64% of researchers who have made their data open could not recall what licensing rights they had granted on the data (e.g. CC-BY, CC-BY-NC)¹⁴. Additionally, 20% of researchers were unaware whether their funders had an open data policy and most researchers welcomed additional guidance on their funders' openness policies¹⁴, suggesting the need for increased education and support. One facet of the Gates Foundation solution to this problem is a new service called Chronos. The Chronos service guides users through submission to services that are compliant with Gates' policy, automatically pays open access fees, and archives manuscripts on PubMed (<https://youtu.be/lweC1BajBBY>). The Gates Foundation expects to scale Chronos to additional funding organizations (<https://chronos.gatesfoundation.org/dynamic.aspx?data=article&key=13-What-is-Chronos&template=ajaxFancyArticle>).

The leadership of funders has led several journals to allow authors to self-archive manuscripts on preprint servers, such as *arXiv* or *bioRxiv*, before publication. Some journals, such as *PeerJ*, also have their own pre-print option⁴⁶. PubMed Central and European PubMed Central also provide open full text archives. The precedent set by these large funders has established an infrastructure and leadership base that smaller funders may be able to leverage in the development and advancement of their own open access policies. Supported by the Laura and John Arnold Foundation, the Center for Open Science also has developed implementation guidelines for funders interested in establishing transparency and openness policies³⁹. Like the TOP journal guidelines, the TOP funder policies are tiered to allow funders to implement more stringent standards over time. Starting in March 2017, the U.S. NIH has begun encouraging investigators to cite preprints or draft (non-peer-reviewed) manuscripts as part of their funding applications⁴⁷.

Box 4. Enhanced open access to data and methodologies

Both governmental and private funders have undertaken significant policy changes to mandate open access to data sets and publications. Funders are generally moving towards more open access, mandating or encouraging researchers to publish in open access journals, paying open access fees, and requiring manuscript archival when researchers publish in more restrictive journals.

Large funders are leading the drive towards open access. NIH spends roughly \$4.5 million on PubMed Central⁴⁵, and requires all grantees to deposit articles and/or manuscripts in this open repository within twelve months of publication (<https://publicaccess.nih.gov/policy.htm>). The Gates Foundation and Howard Hughes Medical Institute have leveraged the NIH's investment by requiring their own grantees to archive manuscripts in PubMed (<http://www.gatesfoundation.org/How-We-Work/General-Information/Open-Access-Policy>, <http://www.hhmi.org/sites/default/files/About/Policies/sc320-public-access-to-publications.pdf>). Gates has gone one step further on open access, requiring all publications to be immediately available in open access "Gold" format (<http://www.gatesfoundation.org/How-We-Work/General-Information/Open-Access-Policy>). The Gates Foundation has also developed tools to assist its grantees with compliance with these new open access policies (<https://youtu.be/lweC1BajBBY>).

As major funders increasingly mandate open access, more journals are providing open access options for authors. Many journals provide Creative Commons copyright options, providing a uniform set of standards. The increased adoption of Creative Commons licenses by journals, especially unrestricted CC-BY licenses, reduces the barrier to adoption of open and transparent sharing permissions (<https://creativecommons.org/licenses/by/4.0/>).

Data standards. Policies that ensure open access to the original underlying data and materials can be leveraged more effectively when the data from different studies can be compared easily. Common standards have been incorporated into reporting policies for journals. For example, the Addgene Vector Database provides a repository of published and commercially-available expression vectors (<https://www.addgene.org/vector-database/>). At least 31 journals recommend or require authors to submit their plasmids to the Addgene repository (<https://www.addgene.org/deposit/pre-publication/>). Addgene performs sequencing to verify submission quality (<https://help.addgene.org/hc/en-us/articles/206135535-What-type-of-Quality-Control-does-Addgene-perform->), and requires each contributor to provide the same types of information in a uniform format, making the database easily searchable and comparable.

The Addgene approach works well for plasmids, which consist of a relatively limited number and size compared to high-throughput, whole genome sequencing data sets. As next generation techniques become more widespread, data standards will become even more important. These data standards include metadata (i.e., information about the data set), data fields, and file formats. With data standards, large data sets become much easier to download and interpret, because users do not have to spend valuable and expensive computational time modifying existing analysis tools to fit each new data set. Researchers have proposed a series of metadata checklists for high-throughput studies⁴⁸. Similar to the development of reagent standards described above, updated data standards will require

multi-stakeholder collaboration within the community of practice, harnessing existing standards where possible and harmonizing divergent practices where appropriate.

Post-publication review. Scientific review is an ongoing process that continues well after peer-review and publication. The broader scientific community may identify issues that were not highlighted by the peer reviewers, and other researchers may attempt to reproduce a study on their own. As the post-publication review process may require experimentation, it warrants dedicated resources.

Despite the time commitment and added value to science, the research community typically does not reward post-publication review. Historically, funding agencies and tenure boards do not tend to reward results reproducibility studies, and researchers can have trouble convincing journals to review and accept such manuscripts. However, stakeholders from different sectors now are dedicating resources to results reproduction. The Laura and John Arnold Foundation currently is funding a cancer biology results reproducibility study as part of its Reproducibility Project series. The first five attempts to reproduce papers as part of this effort were published in January 2017 in the journal *eLife*, an open access journal supported by the Howard Hughes Medical Institute, Max Planck Gesellschaft, and the Wellcome Trust⁴⁹. Two of these five studies successfully reproduced the original findings, one study did not, and two attempts were inconclusive. Since the project seeks to reproduce approximately 50 papers, conclusions about the Project's reproducibility rates at this early stage (i.e., after five experiments) would be premature. An earlier project, Reproducibility Project: Psychology, attempted to reproduce 100 original psychology findings, successfully reproducing one-third to one-half of the results⁵⁰. Another open access publication, *F1000Research*, established the *Preclinical Reproducibility and Robustness Channel* as a platform dedicated to reproducibility of published papers (<https://f1000research.com/channels/PRR>).

Researchers attempting to raise concerns to editors about irreproducible or incorrectly analyzed results found in published articles describe many barriers to the process of raising these concerns, including lack of clarity and transparency from journals in the post-publication review process⁵¹. Similarly, journals do not always have a clearly-defined retraction process that mirrors the submission and peer review processes. Much like the stakeholder discussions on study design, cell line authentication, and open access, the retraction process is an important topic that warrants engagement by the research community. The Committee on Publication Ethics has established best practices for Retraction Guidelines⁵², which may provide an opportunity for this discussion.

Websites, like [PubMed Commons](#) and [PubPeer](#), provide an informal mechanism to facilitate post-publication review and results reproduction attempts by providing a discussion forum for researchers to openly discuss scientific publications. Discussions on these platforms can occur much faster than the pace of published technical commentaries in journals, and provide opportunities for more scientists to contribute. Last year, researchers undertook a widespread deployment of the automated statcheck algorithm

on nearly 700,000 experiments from over 50,000 papers, and automatically generated comments on PubPeer for each paper⁵³. This automated tool helps researchers identify papers that deserve further review and discussion about solutions, such as retraction or publication of counter studies. Discussions on open blogs are a double-edged sword. Whereas rapid turnaround and informal discussion can stimulate productive scientific debate, unmoderated discussion can also lead to unwarranted criticism of legitimate studies. In contrast, technical commentary in journals is refereed by an editor who can help organize and moderate the discussion.

The sheer volume of published research increases the difficulty of identifying and tracking publication errors. Science journalism is another tool that can improve reproducibility. Science reporters, such as the authors of Retraction Watch (www.retractionwatch.com), bring publicity to reproducibility and retraction news, which can galvanize the scientific community to action. For example, replicability of the initial paper describing the NgAgo genome editing technique has been the subject of fierce debate in the community wherein researchers described their difficulties in reproducing the paper's claims on internet and scientific news sites. The technique drew so much attention that over 100 researchers attempted to reproduce the technique in the first few months after publication, but less than 10% were successful⁵⁴. The controversy resulted in three peer-reviewed publications, all of which documented a failure to reproduce the original study, and researchers now are trying to understand the reasons for irreproducibility⁵⁵.

Retraction Watch also partners with the Center for Open Science to generate a database of retractions, as some retracted articles still are cited frequently after retraction⁵⁶. Researchers armed with this database can avoid using retracted work as a (shaky) foundation for new studies, thereby increasing their chance of success. By reading about reproducibility and retraction news, researchers can learn about the common pitfalls that can cause retractions and new resources available to help them improve the reproducibility of their work, such as the initiatives described in this report. However, highly-visible retractions are a potential threat to public confidence and support for science, as the lay public reads more about retractions and irreproducibility. This further highlights the urgent need for the scientific community to act on the initiatives described in this report and make meaningful improvements to reproducibility.

Conclusion: a path forward

Irreproducibility is a serious and costly problem in the life sciences. Measured reproducibility rates are shockingly low, requiring significant effort to solve this problem. Many stakeholders now recognize the importance of reproducibility and are taking steps to develop and implement meaningful policies, practices, and resources to address the underlying issues. The lessons learned from these early efforts will assist all stakeholders seeking to scale up or replicate successful initiatives. The research community is making progress to improve research quality. By prioritizing the strategies outlined in the Report, stakeholders in life science research will continue to make progress in improving reproducibility and in turn have

a profound positive impact on the subsequent development of treatments and cures.

However, the authors would be remiss if we ignored a transcending challenge facing the research community and their willingness to voluntarily accept these positive steps in addressing reproducibility: the current rewards system in academia, including constant pressure to obtain grants and publish in “high impact” journals. The research culture, particularly at academic institutions, must seek greater balance between the pressures of career advancement and advancing rigorous research through standards and best practices. We believe that the many initiatives described in this Report add needed momentum to this emerging culture shift in science, but

additional leadership and community-wide support will be needed to better align incentives with reproducible science and effect this change.

Continued transparent, international, multi-stakeholder engagement is the way forward to better, more impactful science. GBSI calls on all stakeholders – individuals and organizations alike – to take action to improve reproducibility in the preclinical life sciences by joining an existing effort, replicating successful policies and practices, providing resources to results reproduction efforts, and/or taking on new opportunities. **Table 3** contains specific actions that each stakeholder group can take to enhance reproducibility.

Table 3. Reproducibility2020 action plan.

Stakeholder	Actions to improve reproducibility in preclinical research
Funders	<ul style="list-style-type: none"> • Enact policies requiring study design pre-registration, cell line authentication and reagent validation, laboratory protocol transparency, and open access to publications. Provide relevant funding commitments where necessary • Include specific line items in grant review to score reproducibility factors • Provide resources for study design training and statistics consultation for grantees and grant applicants • Fund the development of open access and transparency tools, and additional research to better characterize reproducibility • Fund the development of new technologies and methods that enhance reproducibility • Encourage grantees to develop communities of practice for protocol sharing and testing, and dedicate resources to facilitate and incentivize these communities • Fund innovative training programs including online modules
Researchers and Research Institutions	<ul style="list-style-type: none"> • Make online accessible training modules available that address all major components and evolving approaches of the research process • Explore new approaches to mentorship and accountability to ensure that emerging researchers (i.e., graduate students and postdocs) receive necessary training and supervision from experienced PIs • Implement lab policies that improve reproducibility, such as reagent validation and documentation, routine cell line authentication, and independent reproduction of results by another researcher in the lab • Develop institutional policies and an organizational culture that values and rewards reproduction studies, study design pre-registration, protocol sharing, and open access • Organize online communities of practice to facilitate discussion and sharing of information within the field • Participate in multi-stakeholder groups that develop reproducibility policies and guidelines • Explicitly consider reproducibility issues during peer review of grants and manuscripts • Develop new technologies and methods that improve reproducibility and assist in validation and authentication processes • Explore new technologies including lab/bench automation and robotics to ensure greater precision and minimize errors • Perform results reproduction studies and publish the results • Explore new incentive structures for career advancement that move away from the traditional impact factor and funding paradigms to reward greater data and methods transparency, adherence to best practices and standards, and reproducibility of published work
Journals	<ul style="list-style-type: none"> • Adopt more stringent reporting and transparency guidelines, such as TOP Level 3 • Provide cost-effective open access publication options under CC-BY licenses • Require cell line authentication and promote antibody validation guidelines, as they become available. • Allow archiving of submitted manuscripts before publication • Publish reproduction studies and technical commentary • Consider pre-registered review models that enable rigorous peer review of study design • Encourage greater use of pre-print platforms • Work with researchers to establish data and metadata standards for reporting (e.g., next-generation sequencing) • Require authors to link to version-controlled protocols • Conduct surveys of researchers to better understand reproducibility issues and obtain feedback on journal guidelines and policies • Report on reproducibility issues in the editorial and news section of the journal

Stakeholder	Actions to improve reproducibility in preclinical research
Industry	<ul style="list-style-type: none"> • Transparently communicate the results of in-house replication attempts • Enhance protocol transparency, discussion, and version control, especially for reagents and kits • Provide validation data and technical support for reagents and kits • Participate in the establishment of materials standards
Nonprofits/Scientific Societies	<ul style="list-style-type: none"> • Convene multidisciplinary groups to establish relevant standards, including materials standards for commonly-used reagents, and data standards for commonly-used experimental methods • Provide professional development for researchers to improve research proficiencies, particularly in the areas of as study design, data analysis, reagent validation, and reporting transparency • Convene meetings focused on reproducibility to facilitate sharing of best practices and develop new policies and procedures
Public	<ul style="list-style-type: none"> • Stay aware of reproducibility news to promote a culture of accountability

In its leadership role, GBSI will:

- work with journals and funders to encourage policies that increase rigor, accountability and open access to data and methodologies;
- lead the effort toward improving the validation of reagents—particularly cells and antibodies— and work with the research community to explore other scientific areas (e.g. stem cells and synthetic biology) where a greater emphasis on development of standards and best practices are needed to ensure quality and advance discovery;
- ensure high quality, accessible online training modules available to both emerging and experienced researchers who are eager to improve their proficiencies in new and evolving best practices; and
- continue to track reproducibility efforts through the Reproducibility2020 Initiative.

The preclinical research community is full of talented, motivated people who care deeply about producing high-quality science. We are optimistic about the potential to improve reproducibility, and look forward to contributing to the effort.

Author contributions

LF, GV, and RW conceived of the review study. LF and RW developed the initial outline and GV carried out most of the literature review and completed the first draft. All authors were involved in subsequent revisions of the manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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Lenny Teytelman

protocols.io, Berkeley, CA, USA

This is a carefully considered, well written, and comprehensive overview of the numerous causes of irreproducibility and the many ongoing efforts to address them. This manuscript also provides a set of useful actionable recommendations for researchers, funders, journals, and other stakeholders to improve the rigor and reproducibility of research.

Below are specific comments that I hope the authors will find useful for revising and improving their paper.

1. ATCC is one of the main **fundors of GBSI** and this report mentions ATCC a couple of times. The mentions are appropriate, but the GBSI/ATCC relationship should be clearly disclosed in the COI.
2. [Abstract and Introduction]

Both the abstract and introduction mention the 2012 Amgen report as the beginning of attention to reproducibility. Without a doubt, the Amgen and Bayer headlines have led to a spike of attention and discussion; however the reproducibility issue is not a new problem. Inability to repeat the work of others is as old as science itself and much has been previously written regarding this issue (examples: <https://www.ncbi.nlm.nih.gov/pubmed/16510544>, <http://www.the-scientist.com/?articles.view/articleNo/16604/title/Microarray-Data-Stands-Up-to-Scr>, <http://www.nature.com/ng/journal/v41/n2/full/ng.295.html>, <http://iai.asm.org/content/78/12/4972.full>, <http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0040028>, <http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0020272>, http://elpub.scix.net/data/works/att/001_elpub2008.content.pdf). Moreover, many efforts to improve reproducibility are significantly older than 2012 (for example, Current Protocols, Open Wet Ware, Nature Protocol Exchange, JOVE, and more). Would be good to explicitly acknowledge this.
3. [Introduction] *“Based on these findings, GBSI completed an economic study in 2015 and estimated that the prevalence of irreproducible preclinical research exceeds 50%, with associated annual costs of approximately \$28B in the United States alone[4].”*

As has been publicly discussed after the PLOS Biology publication [4], the estimate of \$28B cost of irreproducible research is on shaky ground (see [The Sensational vs. the Useful in the Quest for Reproducibility in Research](#) and [Study claims \\$28 billion a year spent on irreproducible biomedical research](#)). It extrapolates to all of US Biomedical Funding from a few estimates of irreproducibility

in specific fields. I know of no quantitative research that evaluates reproducibility of published basic research in zebra fish or drosophila communities. If reproducibility problems are greater in cancer, human cell lines, and other research fields, the overall scale of the reproducibility problem across all biomedical research could be smaller.

Also, I very much appreciate the authors' note that the "irreproducible" definition is tricky and that they include results, methods, and inferential reproducibility in their analysis. So, the results may be simply "hard to reproduce" due to missing details or reagents, but they would be included in the "irreproducible total". The definition issues further complicate the attempt to estimate in dollar amounts the scale of irreproducible research.

Instead of saying, "the prevalence of irreproducible preclinical research exceeds 50%, with associated annual costs of approximately \$28B in the United States alone", I urge the authors to simply refer to their publication with something more general such as, "GBSI's 2015 economic study highlighted the high level of economic costs from poor reproducibility."

4. [Study design and analysis]

Box 2 recommends online training courses as highly cost-effective. It is true that they are cost effective, but are they effective when it comes to improving study design? Given how busy scientists tend to be, it is unclear that they will actually devote time to watching online training videos. (For example, podcasts for scientists tend to be consumed much more readily than videos of the same length, as people can listen during commute, runs, cooking, etc. In contrast, videos longer than 3-4 minutes are barely watched by anyone to the end.)

5. [Laboratory protocols]

This section should probably mention the [Protocol Exchange](#) from *Nature/Springer* which is a protocol repository that was started over a decade ago to improve the reporting of methods.

The authors might also want to include a mention of Bio-protocol, a journal devoted to increasing reproducibility. Though a selective peer-reviewed journal rather than a repository, Bio-protocol is also connecting to journals and [eLife recently included them](#) in their author guidelines to encourage scientists when appropriate to submit new method details to Bio-protocol in parallel with their eLife manuscript submission.

6. [Reporting and review]

In the data reporting section, I recommend adding a brief discussion of data repositories such as [Dryad](#) and [figshare](#). Journal policies regarding data sharing are critical and this overview of the genomics community journal policies from Heather Piwowar and Wendy Chapman is relevant: http://elpub.scix.net/data/works/att/001_elpub2008.content.pdf.

Also, the explicit [data policy](#) from the Public Library of Science is an important step in improving reproducibility of published work.

Related to the data policies, sharing code and software from computational pipelines used to

analyze the data is critical. Perhaps add a mention of policies encouraging proper reporting and sharing of code/software?

7. [Reporting and review]

There are important experiments happening with open review from publishers such as F1000 Research, EMBO, BMJ, PeerJ and others. Transparent publication with review/author response history can be helpful for reproducibility as readers can see reviewers' concerns and that can help to discern which parts of the paper are more or less trustworthy.

Another relevant proposal is for the adoption of CRediT (Contributor Roles Taxonomy) by publishers. (See [Transparency In Authors' Contributions And Responsibilities To Promote Integrity In Scientific Publication](#).)

8. [Reporting and review: open access policies]

This section does a good job of summarizing open access initiatives and policies from funders, but the link to reproducibility is unclear. As an advocate for open access, I am delighted to see these developments, but the connection between open access publishing and increased reproducibility is not obvious to me.

A paper in a subscription journal can be solid and reproducible, while one in an open access journal is not. The reverse is just as likely. Certainly, this is more a function of chance and editorial and peer review vigilance than the journal's business model.

An argument can be made for how open access enables reproducibility initiatives (ex. CiteAb), but I don't think I saw it in this paper.

9. [Reporting and review: preprints]

As above for open access, I am a huge fan of preprints but am unsure how they fit into the push for greater reproducibility. Preprints, of course, shorten publication delays, facilitating communication and speeding up research. However, preprints are not peer-reviewed, do not go through conflict-of-interest checks, data/method reporting compliance checks, and so forth. At scale adoption of preprints in biology is welcome for many reasons, but not exactly due to more rigor and higher reproducibility.

(Possibly, preprints reduce the pressure to publish and create a track record of a paper's initial state, reducing publication biases? Preprints can also help to challenge previously-published work and to report negative results. If these are the arguments for preprints improving reproducibility, please make this case explicitly in the manuscript.)

(Minor note: the use of "preprint" versus "pre-print" is inconsistent in this paper. Please remove the extra dash.)

10. [Table 3, action plan]

For funders, there is a recommendation to "*Enact policies requiring study design pre-registration*". I am on the steering committee for COS's pre-registration initiative and support this effort, but I am

not sure that “requiring” pre-registration widely is appropriate. This will depend on the funder and specific research grant. For example, in the case of method development and highly explorative grants, pre-registration is unlikely to be productive. How about “encourage where appropriate” instead of “require”?

For journals, there is a recommendation to “*Require authors to link to version-controlled protocols*”. Again, “require” is a strong term. In certain cases, it may be better to share a protocol directly as part of the publication (for example, JOVE). A more general “encourage or require detailed reporting of protocols” may be more appropriate.

11. [Conclusion] *“Irreproducibility is a serious and costly problem in the life sciences. Measured reproducibility rates are shockingly low, requiring significant effort to solve this problem.”*

I very much agree with the first sentence in that irreproducibility is a serious problem. However, is the reproducibility rate “shockingly” low? What is that rate for biology in general? As discussed above, 50% may be the number for some fields but not for others. More importantly, what rate are we aiming for? 70%? 90%? If all of the action items recommended in this report were followed, what rate would we end up with? Is our current level of reproducibility better or worse than it was 30 years ago? What is the optimal reproducibility rate from society’s perspective?

I don’t have the answers to the above questions. We need a lot more data to make informed statements about the levels of reproducibility over time. It is terrific that we are discussing this issue and the initiatives to address the problem, but I urge caution in editorializing about whether today’s reproducibility levels are a “crisis” or are “shocking”. Science is hard and because it is pushing the boundaries of knowledge, we will never be at 100% of published research being reproducible. We can and should do a lot better, hence all of the initiatives, but it will never be 100%.

12. [General thoughts]

As I mention in #11 above, with the exception of a few efforts from Science Exchange and the Center for Open Science, we have very little data on the reproducibility issue. The authors may want to include in their discussion the need for more quantitative studies about replication and reproducibility over time. We need ways to assess the various initiatives and to measure whether they are in fact improving the overall reproducibility levels of published research.

Also, most of the recommendations and discussion in this Report are focused on the design, execution, and publication steps of the research cycle. However, given the complexity of research and the fact that we will never attain 100% reproducibility, efforts aimed at post-publication opportunities to improve reproducibility may be particularly effective. Perhaps we should pay more attention not just to preventing mistakes, but to ways to correct and improve papers, long after publication.

This Report mentions post-publication review and retractions, but there are other promising efforts in this phase. Versioning, as implemented on F1000Research and bioRxiv, has great potential. There is a need for technologies that automatically connect readers to corrections and discussion on the papers that they have in their libraries. Crossmark from Crossref is a great initiative aimed at making corrections discoverable. Also, an interesting [recent proposal](#) argues for rethinking of “retractions/corrections” in favor of “amendments” to increase post-publication evolution and improvement of work.

I would like to stress that I thoroughly enjoyed this report and am grateful to the authors and GBSI for their efforts to improve the research enterprise for the benefit of scientists and the public. The authors should feel free to ignore any of the above suggestions if they disagree.

Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: COI: I am the CEO and co-founder of the protocol repository protocols.io, which is positively mentioned several times in the current manuscript. I am also on the steering committee for GBSI's 2017 reproducibility symposium and GBSI has previously used protocols.io for hosting reproducibility discussions regarding antibodies.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Michael S. Lauer

National Institutes of Health (NIH), Bethesda, MD, USA

Freedman and colleagues present a narrative review on current efforts underway to improve reproducibility in preclinical biomedical research. They begin by summarizing the extent of the problem and noting that quality checkpoints are either used in disparate points of the research cycle or used only sparingly. They identify key sources of irreproducibility as poor study design and analysis, inadequately authenticated reagents and reference materials, inadequately documented laboratory protocols, and inadequate reporting and review. They describe the important roles of many stakeholders, including funders, researchers, research institutions, industry, foundations, professional societies, and the public.

The authors proceed to describe many efforts already under way including new funder requirements, journal guidelines, enhanced training opportunities, programs to enhance standards development and authentication checks, protocol repositories, improved reporting platforms, open access policies (including open access publishing, greater use of preprint servers, data and code sharing), data standards, and post-publication review. They conclude with a "path forward" that they call the "Reproducibility2020 Action Plan" that includes specific recommendations for funders, researchers, institutions, journals, industry, foundations, and the public.

Thoughts and comments:

- The paper is interesting, well-written, and well-documented. I appreciated the many web links that take the reader directly to interesting sites.
- The authors suggest that the current crisis begins with the Amgen findings (Reference 2). While that was a defining moment, I wonder whether it's also worth mentioning that contemporary discussion about false research findings dates back at least as far back as Ioannidis 2005 (<https://doi.org/10.1371/journal.pmed.0020124>). Ioannidis there suggests that exploratory research was highly vulnerable because of small sample sizes, overly flexible designs, and biased designs (e.g. with lack of randomization and proper masking).
- Table 1: I commend the authors for noting that “the chance of an irreproducible finding is much higher than the commonly noted 5% threshold.” This is widely under-appreciated, even by well-trained scientists. The authors might consider spelling out that prospective, properly done sample size calculations are critical to overcoming this problem. The “elephant in the room” is that sample sizes will have to increase substantially, meaning that with constrained funds researchers will be forced to conduct fewer experiments. But as some have noted (Cressey D, *Nature*, April 15, 2015), that may be good for the enterprise – it would be better to do fewer properly powered experiments than to do too many woefully underpowered experiments.
- Table 1 and elsewhere: Should there be a “Consumer Reports” for antibodies, cell lines, and other resources? Or maybe I’m missing it, and you’re saying that’s happening. Such a “Consumer Reports” would allow for large-scale surveys in which researchers can report problems with purchased materials.
- Table 1: Another potential solution to study design and analysis is mandatory sharing of statistical code (e.g. in SAS, R, or Stata). This is already common practice in some fields (e.g. economics).
- Table 2: Another consequence for the public is lack of faith in science. They hear scientists promising the moon, and then nothing happens.
- Table 2: There is an ethical problem subjecting animals and people to inadequately designed or documented experiments that were doomed to be irreproducible from the beginning.
- Table 2 or elsewhere: NAS just released a report on research integrity in which notes a continuum between frank misconduct (fabrication, falsification, and plagiarism) and “practices detrimental to research.” The authors might want to consider the comments of the report (<https://www.nap.edu/catalog/21896/fostering-integrity-in-research>).
- There have been some recent successes in improved rigor, such as in preclinical stroke research. (For example, see <http://circres.ahajournals.org/content/early/2017/04/04/CIRCRESAHA.117.310628>). The authors note that “stroke research has uniquely improved.”
- Page 6 – the link didn’t take me directly to “Statcheck software,” though I did eventually find it.

- Protocols – many leading clinical journals require authors to submit full clinical trial protocols along with the manuscripts.
- Table 3
 - Should it be the responsibility of funders to provide statistical consultation to applicants?
 - Should it be the responsibility of funders to pay for open access and transparency tools?
 - Should funders include dedicated reviews on methodological issues for those applications deemed meritorious by content?

Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
